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Biosynthesis « De Novo » of the Ophuirid Ophiocomina Nigra Igkappa Gene

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Abstract

A biosynthesis « de novo » of the ophuirid Ophiocomina nigra IGKappa gene was performed in our laboratory. This gene was inserted into pUC-GW plasmid by using the unique restriction site. The synthetized nucleotide sequence was identical to the original one.

We concluded to the validity of the experiment.The unique ancestral IGKappa gene and the corresponding protein (IGKappa) possesses immunoglobulin sites

Keywords: IGKappa; pUC-GW

Abbreviations: PCA: poly chain assembly method, DNA: Deoxyribonucleic acid.

Introduction:

In recent papers **[1]** we have presented the sequence of Ophiocomina nigra IGKappa gene as following (in 5'- 3'): It presented 1019 bp.

BC030813.1

GAGGAACTGCTCAGTTAGGACCCAGACGGAACCAT
GGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACT
CTGGCTCCCAGATACCACTGGAGAAATAGTGATGA
CGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGG
AAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGT
GTTACCAGCAACTTAGCCTGGTACCAGCAGACACC
TGGGCAGTCTCCCAGGCTCGTCATCTATGGTGCATC
CAGCAGGGCCAGTGGTGTCCCAGCCAGGTTCAGTG
GCAGTGGGTCTGGGACAGAGTTCACTCTCACCATC
AGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTAC
TGTCAGCAGTATAATAAGTGGCCGCACACTTTTGG
CCAGGGGACCAAGCTGGACATCAAACGAACTGTGG
CTGCACCATCTGTCTTCATCTTCCCGCCATCTGATG
AGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCC
TGCTGAATAACTTCTATCCCAGGGAGGCCAAAGTA
CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAG
GACAGCACCTACAGCCTCAGCAGCACCCTGACGCT
GAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGA
GGGAGAAGTGCCCCCACCTGCTCCTCAGTTCCAGC
CTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTT
CCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCT
CATCTTTCACCTCACCCCCCTCCTCCTCCTTGGCTTT
AATTATGCTAATGTTGGAGGAGAATGAATAAATAA
AGTGAATCTTTGCAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

We have tried, in the present paper, to synthetize the corresponding gene « de novo », according to the method we describe now:

Materials and methods:

We have used the following gene synthesis method:

1. Synthesis of oligonucleotides with overlapping segments in sense and antisense direction
2. Assembly of the oligonucleotides into a double stranded DNA, using a poly chain assembly method (PCA).
3. For larger constructs, the sequence is split into smaller, intermediate fragments, to facilitate synthesis. Once the intermediated fragments have been obtained with correct sequence, they are assembled into the full-length sequence.
4. Cloning into the linearized vector by either recombination or ligation based cloning, mostly performed within the same step as full-length sequence assembly.

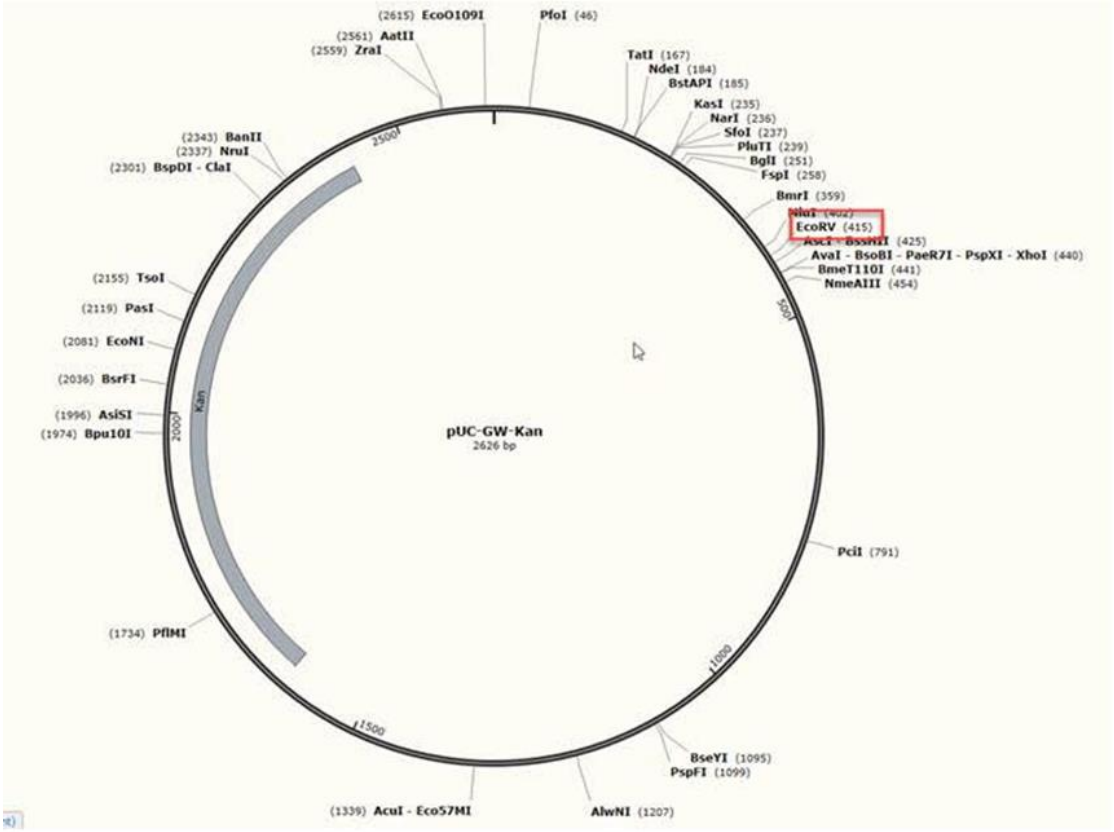
Regarding the restriction site, which was used for cloning, construct BC030813.1 was cloned into vector pUC-GW by using the unique EcoRV restriction site. The final construct sequence was achieved after use of primers **(Table 1)**.

M13F-77	GATGTGCTGCAAGGCGATTA
M13R-88	TTATGCTTCCGGCTCGTATG
U-SEQ4883	CCTCCAATCGGGTAACTC

Table 1: Primers used for sequencing

Results:

Plasmid map:



Original sequence and synthetised one were performed into the plasmid. Chromatograms assert them.

Original sequence:

GAGGAACTGCTCAGTTAGGACCCAGACGGAACCAT
GGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACT
CTGGCTCCCAGATACCACTGGAGAAATAGTGATGA
CGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGG
AAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGT
GTTACCAGCAACTTAGCCTGGTACCAGCAGACACC
TGGGCAGTCTCCCAGGCTCGTCATCTATGGTGCATC
CAGCAGGGCCAGTGGTGTCCCAGCCAGGTTCAGTG
GCAGTGGGTCTGGGACAGAGTTCACTCTCACCATC
AGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTAC
TGTCAGCAGTATAATAAGTGGCCGCACACTTTTGG
CCAGGGGACCAAGCTGGACATCAAACGAACTGTGG
CTGCACCATCTGTCTTCATCTTCCCGCCATCTGATG
AGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCC
TGCTGAATAACTTCTATCCCAGGGAGGCCAAAGTA
CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAG
GACAGCACCTACAGCCTCAGCAGCACCCTGACGCT
GAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGA
GGGAGAAGTGCCCCCACCTGCTCCTCAGTTCCAGC
CTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTT
CCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCT
CATCTTTCACCTCACCCCCCTCCTCCTCCTTGGCTTT
AATTATGCTAATGTTGGAGGAGAATGAATAAATAA
AGTGAATCTTTGCAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Synthetized sequence:

GAGGAACTGCTCAGTTAGGACCCAGACGGAACCAT
GGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACT
CTGGCTCCCAGATACCACTGGAGAAATAGTGATGA
CGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGG
AAAGAGCCACCCTCTCCTGCAGGGCCAGTCAGAGT
GTTACCAGCAACTTAGCCTGGTACCAGCAGACACC
TGGGCAGTCTCCCAGGCTCGTCATCTATGGTGCATC
CAGCAGGGCCAGTGGTGTCCCAGCCAGGTTCAGTG
GCAGTGGGTCTGGGACAGAGTTCACTCTCACCATC
AGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTAC
TGTCAGCAGTATAATAAGTGGCCGCACACTTTTGG
CCAGGGGACCAAGCTGGACATCAAACGAACTGTGG
CTGCACCATCTGTCTTCATCTTCCCGCCATCTGATG
AGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCC
TGCTGAATAACTTCTATCCCAGGGAGGCCAAAGTA
CAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAG
GACAGCACCTACAGCCTCAGCAGCACCCTGACGCT
GAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGA
GGGAGAAGTGCCCCCACCTGCTCCTCAGTTCCAGC
CTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTT
CCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCT
CATCTTTCACCTCACCCCCCTCCTCCTCCTTGGCTTT
AATTATGCTAATGTTGGAGGAGAATGAATAAATAA
AGTGAATCTTTGCAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

We give now the Blastn original sequence/ synthetized sequence results:

Size Seq1	Size Seq2	Max score	Total score	Query cover	E. Value	Per. Ident	Acc Len
1019	1019	1882	1882	100%	0.0	100%	1019

Conclusion Discussion:

The synthesized nucleotide sequence is identical to the original sequence. It is therefore valid.

In conclusion, we have obtained with success an IGKAPPA Gene with its unique IGKAPPA protein. Additionally, the existence of members of IGKappa genes with IG sites in Invertebrates and particularly in Echinodermata **[2]** (Ophuirids, Asterids) constitute an excellent opportunity to explore relations of molecular structures and biochemical and physiological function of these unique proteins.

References:

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2) Vincent N, Osteras M, Otten P, Leclerc M (2014) A new gene in A. rubens: A sea star Ig kappa gene. Meta Gene. 2: 320-322.